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Enhancing viability of two biocontrol yeasts in liquid formulation by applying sugar protectant combined with antioxidant

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Abstract Survivals of *Cryptococcus laurentii* and *Pichia membranaefaciens* in liquid formulations with sugar protectants (trehalose and galactose) and *L*-ascorbic acid (Vc) were investigated during storage at 4°C and 25°C. When galactose or trehalose was used alone as protectant, *C. laurentii* maintained relatively high viability in potassium phosphate buffer. Addition of Vc to trehalose improved its protective effect. *P. membranaefaciens* maintained viability >60% after 90 days at 4°C when 5% galactose served as a protectant, and its combination with Vc was the most effective at maintaining viability. Moreover, liquid formulation kept higher viability of the two yeasts at 4°C than at 25°C. Biocontrol efficiency of the two yeasts was maintained after formulation and storage. The results indicate that trehalose is considered as a suitable protectant for liquid formulation of *C. laurentii*, while galactose is better for *P. membranaefaciens*. Combining Vc with the sugars improves the protective efficiency.

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Introduction

Postharvest diseases caused by fungal pathogens result in major economic losses worldwide. Synthetic fungicide treatment has been the main method to control fungal pathogens of fruits. However, biological control using microbial antagonists has shown potential as an alternative (Spadaro and Gullino 2004). Antagonistic bacteria have shown biocontrol efficacy against pathogens of fruits like apple, citrus, and pear (Janisiewicz and Jeffers 1997; Nunes et al. 2001; Teixidó et al. 2001), and antagonistic yeasts are effective in controlling postharvest diseases of fruits like apple, citrus, peach, pear, strawberry, and sweet cherry (Droby et al. 1998; Helbig 2002; Lima et al. 2003; Xu and Tian 2008; Xu et al. 2008; Yao et al. 2004; Zhang et al. 2009). By comparison with antagonistic bacteria, yeasts have been pursued actively in recent years, as production of antibiotics or other toxic secondary metabolites is not involved in their activities against postharvest pathogens.

Commercial formulation of biocontrol yeasts should possess adequate shelf life and retain biocontrol efficacy. Dry and liquid formulations are two successful methods commonly used for preserving biocontrol agents. In general, the advantages of dry

formulation are storage for longer period and at higher temperatures, protection from contamination or infestation during storage, and ease of strain distribution (Li and Tian 2006; Melin et al. 2007). However, the relatively high cost of drying technology makes liquid formulation of biocontrol yeasts as an alternative. Moreover, liquid formulation has an advantage over dry formulation since it does not need to be hydrated and rehydrated, during which high mortality often occurs (Eleutherio et al. 1993; Li and Tian 2006). Previous studies have indicated that certain liquid formulations of the biocontrol yeasts *Candida sake* (Abadias et al. 2003; Torres et al. 2003), and *Pichia anomala* (Melin et al. 2006) with high viabilities of cells were phosphate buffers supplemented with either trehalose or lactose.

Cryptococcus laurentii and *Pichia membranaefaciens* are two biocontrol yeasts commonly used in the control of postharvest diseases on various fruits, such as apple, peach, orange, and sweet cherry (Xu and Tian 2008; Xu et al. 2008; Yu et al. 2008; Zhang et al. 2005). In previous studies on dry formulation of *C. laurentii* by using a freeze dryer, it was found that trehalose enhanced tolerance of *C. laurentii* to freeze drying stress (Li and Tian 2006) and intracellular trehalose of *C. laurentii* affected its viability (Li and Tian 2007). Recently, Li et al. (2008) reported effects of endo- and exogenous trehalose on viability of *C. laurentii* and *Rhodotorula glutinis* after being treated with rapid freezing, slow freezing, and freeze drying, respectively.

In this study, we mainly approached liquid formulations for *C. laurentii* and *P. membranaefaciens* in low and room temperature storage at laboratory scale by applying sugar protectant combined with *L*-ascorbic acid (Vc) to evaluate the effect on viability. In addition, the biocontrol efficacy of the two yeasts in liquid formulations against *Penicillium expansum* and population dynamics were evaluated on peach fruits.

Materials and methods

Yeasts and culture media

C. laurentii and *P. membranaefaciens* were isolated in our previous experiments and identified by CABI Bioscience Identification Services (International Mycological Institute, UK).

Five hundred milliliters of nutrient yeast dextrose broth (NYDB: 8 g of nutrient broth (Oxoid, UK), 5 g of yeast extract (Oxoid, UK), and 10 g of dextrose (Beihua Fine Chemicals Co., Ltd., China) in 1000 ml water) was prepared in 1-l conical flasks, inoculated with *C. laurentii* or *P. membranaefaciens* to an initial concentration of 10^4 CFU ml^{-1}, and incubated for 48 h at 26°C on a shaker at 200 rpm.

Pathogen

P. expansum was isolated from infected peach fruit and maintained on potato dextrose agar (PDA) (Oxoid, UK) at 4°C. The pathogen was inoculated into peach fruit and re-isolated onto PDA before the experiment. Spore suspension was obtained from two-week-old cultures. The number of spores was calculated with a hemocytometer, and then the spore concentration was adjusted to 5×10^3 spores ml^{-1} with sterile distilled water.

Fruits

Peach (*Prunus persica* (L.) Batsch) fruits were harvested at commercial maturity, and the fruits without wounds or rot were classified according to uniformity of size, disinfected with 2% (v/v) sodium hypochlorite for 2 min, cleaned with tap water, and dried in air prior to use according to the method of Yao et al. (2004).

Evaluation of viability of the biocontrol yeasts in preservation solution with protective substances

Yeast cells were pelleted at 8,000 *g* for 5 min at 4°C and resuspended in 0.05 mol l^{-1} potassium phosphate buffer, pH 6.5 (PPB). This buffer was supplemented with trehalose, galctose at 5% and 10% (m/v) separately, or combined with 0.0176% (m/v) Vc (1 mM). All liquid formulations were prepared in 5×10^8 CFU ml^{-1} of cell suspension, and 5 ml was distributed in 10-ml glass tubes. The initial concentration of viable cells (CFU ml^{-1}) was determined by the plate method on NYDA (NYDA: as for NYDB with addition of 20 g of agar (Shuangxuan, China)); 10-fold dilutions of each yeast strain were spread on NYDA plate. Plates were incubated at 26°C for three days, and the number of colony-forming units

per milliliter (CFU ml^{-1}) was calculated. Samples were incubated at both 4°C and 25°C for 30, 60, 90 days and 5, 10, 15 days, respectively. After different periods of time, the number of viable cells was determined according to the plate method described above. Viability of cells after preservation treatments was expressed as a percentage of surviving *C. laurentii* or *P. membranaefaciens* cells compared with the initial number of cells. There were three replicates in each treatment, and the experiment was repeated twice.

Efficacy of liquid formulations of the biocontrol yeasts against *P. expansum* on peach fruits

Suspensions of both yeast cells with the highest viability percentage in trehalose- or galactose-based liquid formulation after 90 days of storage at 4°C were tested against *P. expansum* on peach fruits. The efficacy of fresh cells grown under the same condition as described above was assayed as well. Cell suspensions of both yeasts were adjusted to 1×10^7 CFU ml^{-1}. Three wounds (4 mm deep × 3 mm wide) were made on the equator of each fruit with a sterile nail. A 20 µl suspension of formulated or fresh cells of *C. laurentii* or *P. membranaefaciens* was applied to each wound, and sterile distilled water served as control. When the fruits were air dried for 2 h, 5 µl of *P. expansum* suspension (5×10^3 spores ml^{-1}) was inoculated into each wound. Treated fruits were placed in plastic box with a polyethylene bag and then stored at 25°C. Disease incidence and lesion diameter caused by *P. expansum* were determined after four days. Each treatment contained three replicates with five fruits per replicate and the experiment was repeated twice.

Population of the biocontrol yeasts in wounds of peach fruit

Peach fruits were treated as described above without inoculation of *P. expansum*. Samples were prepared at different time points after treatment according to the previous study (Fan and Tian 2001). The yeasts were recovered by removing five wound tissues with a cork borer (1 cm diameter × 1 cm deep), ground with a mortar and pestle in 10-ml sterile distilled water. Then, 50 µl of serial 10-fold dilutions were spread on NYDA plates. Samples taken at 1 h after treatment for population measurement served as time 0. Fruits stored at 25°C were assessed every 24 h for 96 h. Colonies were counted after incubation at 26°C for three days and expressed as the Log_{10} CFU per wound. There were three replicates in each treatment, and the experiment was repeated twice.

Data analysis

The results from three independent experiments were accordant, and data from one representative experiment are presented in this paper. All statistical analyses were performed with SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Data were analyzed by one-way ANOVA. Mean separations were performed by Duncan's multiple range tests. Differences at $P < 0.05$ were considered significant.

Results

Effect of protective substances on preservation of *C. laurentii*

In preliminary experiment, we assessed the effects of sugar protectants, such as trehalose, galactose, lactose, sucrose, and glucose on viability of the two yeasts. Trehalose and galactose showed much better protective effect as compared to lactose, sucrose, and glucose (data not shown). Consequently, the two sugars were chosen for the formal study.

The survival of *C. laurentii* cells stored at different temperatures was investigated. After 90 days at 4°C, *C. laurentii* cells in PPB showed viability of 0.7% and those preserved in sugar solutions showed much higher viabilities (Table 1). Generally, trehalose (TRE) provided better protective effect than galactose (GAL) for *C. laurentii*. Addition of Vc to trehalose improved its effectiveness. *C. laurentii* cells in 5% TRE + Vc solution showed the highest viability of 53.4%.

Storage temperature showed notable effect on the viability of the yeast in these liquid formulations. At 25°C, viability of *C. laurentii* in PPB decreased to 0.2% after 15 days. However, all sugars significantly improved viabilities, and Vc addition enhanced the protective effect of trehalose. The treatment of 5% TRE + Vc presented the highest viability of 23.4% after 15 days.

Table 1 Percentage of viable counts of *C. laurentii* liquid formulations stored at 4°C and 25°C

Protectant	Percentage of viable cells of *C. laurentii*					
	4°C			25°C		
	Storage time (days)			Storage time (days)		
	30	60	90	5	10	15
PPB	20.3± ± 1.8e	15.1 ± 2.1f	0.7 ± 0.2e	13.0 ± 2.1d	4.1 ± 0.6e	0.2 ± 0.1e
5% TRE	76.5 ± 4.6c	52.0 ± 3.4c	41.3 ± 2.7b	42.5 ± 3.5c	23.6 ± 3.1c	15.4 ± 3.8bc
5% TRE+VC	87.8 ± 2.2ab	68.1 ± 4.8a	53.4 ± 3.8a	62.4 ± 0.8a	31.4 ± 1.4a	23.4 ± 2.4a
10% TRE	84.3 ± 2.0b	58.9 ± 2.7b	38.3 ± 1.6b	57.5 ± 0.8b	27.5 ± 0.6b	17.8 ± 2.1b
10% TRE+VC	89.4 ± 3.5a	64.2 ± 3.6a	50.2 ± 2.0a	61.4 ± 1.2ab	32.2 ± 1.5a	18.5 ± 1.2b
5% GAL	70.2 ± 1.7d	32.5 ± 0.7e	20.4 ± 2.8d	42.9 ± 3.1c	18.2 ± 1.5d	12.4 ± 0.7 cd
5% GAL+VC	72.3 ± 3.3 cd	37.4 ± 0.6d	22.1 ± 2.3d	43.6 ± 4.7c	16.2 ± 1.6d	10.5 ± 0.4d
10% GAL	72.4 ± 2.4 cd	36.4 ± 1.2de	24.6 ± 2.7 cd	46.4 ± 0.5c	22.3 ± 1.9c	12.8 ± 1.5 cd
10% GAL+VC	75.4 ± 1.2c	40.1 ± 2.5d	27.2 ± 2.1c	44.1 ± 4.2c	21.4 ± 2.4c	14.4 ± 0.5c
df(factor)	8	8	8	8	8	8
df(error)	18	18	18	18	18	18
F-value	175.4	118.7	140.6	87.0	72.4	37.0
P-value	1.8E−15	5.6E−14	1.3E−14	8.5E−13	4.1E−12	1.3E−9

Values are averages of three determinations ± standard deviation. *F*-values, df, and *P*-values are obtained by comparison of the nine average values for each time point by one-way ANOVA, and the values followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$)

TRE Trehalose, GAL galactose

Effect of protective substances on preservation of *P. membranaefaciens*

Similar to *C. laurentii*, viable counts of *P. membranaefaciens* were much higher at 4°C than at 25°C for all protective substances (Table 2). After 90 days at 4°C, all sugar formulations significantly improved viabilities of the yeast, and Vc addition enhanced the effects. Moreover, at the same storage time, galactose provided better protective effect than trehalose for *P. membranaefaciens*. The best results after 90 days of storage at 4°C were obtained in 5% GAL + Vc and 10% GAL + Vc solutions with viabilities of 82.0% and 75.3%, respectively.

After 15 days of storage at 25°C, *P. membranaefaciens* cells in PPB showed viability of 1.7%, and those preserved in sugar solutions showed much higher viabilities, especially in 10% GAL solution. Moreover, the addition of Vc to sugar solutions generally improved the effectiveness. *P. membranaefaciens* cells in 10% GAL + Vc solution showed the highest viability of 82.8%, which was much higher than that of 64.6% in 10% GAL solution.

Biocontrol efficacy and population dynamics of the two yeasts

According to the viability evaluation, cell viability of *C. laurentii* in 5% TRE + Vc and 10% GAL + Vc solutions remained the highest percentage in individual kind of sugar solution after 90 days of storage at 4°C (Table 1), suggesting the prospective efficacy of both liquid formulations. Thus, *C. laurentii* cells in 5% TRE + Vc and 10% GAL + Vc solutions were chosen in the following biocontrol assay. Likewise, 5% GAL + Vc and 10% TRE + Vc, respectively, with the best protective effect in individual kind of sugar solution, were chosen for *P. membranaefaciens* biocontrol assay. The suitable liquid formulations of *C. laurentii* and *P. membranaefaciens* for *P. expansum* control were 5% TRE + Vc and 5% GAL + Vc solutions, respectively (Fig. 1), which corresponded to their best protective effects on cell viability. Disease incidence of peach fruits treated with the two liquid formulations was respectively reduced to 31% and 22%, while that of control fruits reached nearly 100%. Moreover, *P. membranaefaciens* cells in 5%

Table 2 Percentage of viable counts of *P. membranaefaciens* liquid formulations stored at 4°C and 25°C

Protectant	Percentage of viable cells of *P. membranaefaciens*					
	4°C			25°C		
	Storage time (days)			Storage time (days)		
	30	60	90	5	10	15
PPB	92.0 ± 3.1bc	36.7 ± 0.9 g	12.8 ± 2.0 h	65.2 ± 2.7e	6.4 ± 0.6f	1.7 ± 0.3 g
5% TRE	95.5 ± 3.0ab	44.6 ± 3.5f	38.7 ± 3.6 g	81.4 ± 1.8d	7.6 ± 0.8f	5.4 ± 0.5f
5% TRE+VC	97.6 ± 2.1a	59.3 ± 1.7e	43.4 ± 1.5f	90.3 ± 0.9b	15.4 ± 2.0e	12.7 ± 0.7e
10% TRE	90.1 ± 0.8c	60.2 ± 2.3e	49.5 ± 2.9e	88.4 ± 2.5bc	17.4 ± 0.7e	14.4 ± 1.4de
10% TRE+VC	96.6 ± 0.5a	72.3 ± 4.8d	56.3 ± 2.4d	92.2 ± 3.5ab	21.1 ± 2.4d	17.2 ± 0.7d
5% GAL	93.4 ± 3.3abc	87.5 ± 4.6bc	63.0 ± 2.8c	86.3 ± 0.7c	69.3 ± 3.0c	33.4 ± 2.7c
5% GAL+VC	97.6 ± 0.7a	95.4 ± 1.7a	82.0 ± 1.7a	86.2 ± 1.5c	75.4 ± 1.3b	36.3 ± 2.1c
10% GAL	91.4 ± 1.7bc	82.4 ± 3.8c	51.7 ± 3.1e	89.2 ± 1.3bc	77.7 ± 2.0b	64.6 ± 4.2b
10% GAL+VC	93.3 ± 2.8abc	92.3 ± 2.2ab	75.3 ± 0.9b	95.5 ± 2.2a	88.1 ± 1.9a	82.8 ± 2.7a
df(factor)	8	8	8	8	8	8
df(error)	18	18	18	18	18	18
F-value	4.5	139.1	207.4	53.9	1074.8	518.6
P-value	3.8E−3	1.4E−14	4.0E−16	5.3E−11	1.7E−20	1.1E−19

Values are averages of three determinations ± standard deviation. *F*-values, df, and *P*-values are obtained by comparison of the nine average values for each time point by one-way ANOVA, and the values followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$)

TRE Trehalose, GAL galactose

GAL + Vc solution exhibited similar biocontrol effect to fresh cells (Fig. 1b and d).

Population dynamics of the two yeasts in wounds of peach fruit was assessed. As shown in Fig. 2, *C. laurentii* and *P. membranaefaciens* multiplied quickly in the wounds of peach fruits at 25°C. *C. laurentii* preserved in 5% TRE + Vc solution showed higher populations than in 10% GAL + Vc solution, and *P. membranaefaciens* in 5% GAL + VC solution multiplied more quickly than in 10% TRE + Vc solution, while fresh cells did most quickly. Populations of the two yeasts showed marked difference among all the treatments at time 0, but the difference became less over the next 96 h.

Discussion

In the present study, viability of *C. laurentii* and *P. membranaefaciens* in liquid formulation was significantly improved by applying trehalose and galactose as protectants and enhanced by combining with Vc (Tables 1 and 2). It has been well established that sugars can be used as exogenous protectants in liquid and dry formulations of biocontrol yeasts (Abadias et al. 2001; Li and Tian 2007; Melin et al. 2007). In dry formulation, direct interactions between sugar molecules and membrane phospholipids, or sugar molecules and proteins, and vitrification of sugars are thought to be the main protective mechanisms (Crowe et al. 1998). The role played by the external addition of trehalose in protecting yeast cells in liquid formulation is well known. One possible explanation of the protective effect is that trehalose traps proteins by increasing media viscosity (Singer and Lindquist 1998; Sampedro and Uribe 2004). Thus, the cells become more stable when certain proteins are inactivated. Moreover, Salek and Arnold (1995) and Abadias et al. (2003) reported that yeast could have a trehalose-specific carrier in cells. Therefore, external addition of trehalose can be transported and accumulated in the cytosol and have a profound influence on water activity of the cytosol, slowing down the metabolism, and promoting the transition to a resting state of cells. External addition of other sugars, such as galactose in liquid formulation may have similar protective

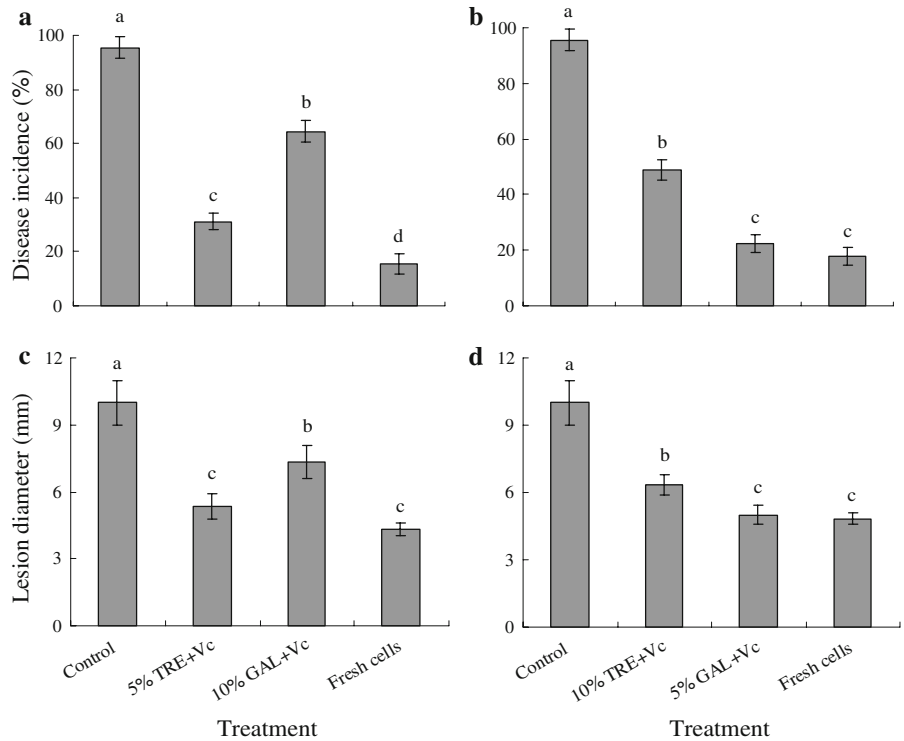

Fig. 1 Biocontrol efficacy of liquid formulations of *C. laurentii* (**a** and **c**) and *P. membranaefaciens* (**b** and **d**) after 90 days of storage at 4°C against *P. expansum* decay on peach fruits. Fruits were wounded and inoculated with 20 µl of sterile water (control), formulated or fresh cells of *C. laurentii* or *P. membranaefaciens* at 1×10^7 CFU ml^{-1}. After 2 h, wounds were inoculated with 5 µl of *P. expansum* at 5×10^3 spores ml^{-1}. Fruits were stored at 25°C for four days after which disease incidence and lesion diameter were determined. Error bars indicate standard deviations. Columns with different letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$). Abbreviations: TRE, trehalose; GAL, galactose

mechanisms. In this study, we noticed that trehalose provided good protective effect, and combining trehalose with Vc showed higher cell viability of *C. laurentii* in liquid formulation (Table 1). However, trehalose did not provide protective effect as good as galactose for *P. membranaefaciens* (Table 2), demonstrating that there are differences in protective effects of sugar on various yeast species.

Oxidative stress may be one of important factors leading to decrease in viability of yeast cells in liquid formulation. Jakubowski et al. (2000) reported that oxidative stress occurred during aging of stationary cultures of *Saccharomyces cerevisiae*, and Patiño-Vera et al. (2005) postulated that during storage of *Rhodotorula minuta* in liquid formulation, accumulation of reactive oxygen species in cells affected the viability. Vc, an effective oxygen scavenger, has been widely applied as a food additive. In the present study, we found that Vc could enhance the effect of sugar protectant on viabilities of biocontrol yeasts *C. laurentii* and *P. membranaefaciens* in liquid formulation, which might be attributed to its antioxidant property of preventing cell damage from oxidative stress. Moreover, natural antioxidants including Vc have the potential activities that lower the microorganisms growth by inhibiting metabolic activity (Ahmad et al. 2005), which may be another possible action mode of Vc in improving the viabilities of the two yeasts in liquid formulation. However, the exact mechanisms behind improvement of viability by Vc still need further study.

High viability of biocontrol yeasts is an advantage in competing for nutrients and space, which plays a major role in biological capability (Piano et al. 1997). In accordance with the results presented by Li et al. (2008), the ability of *C. laurentii* and *P. membranaefaciens* to colonize the wounded fruits was positively correlated with their viability, especially in the first

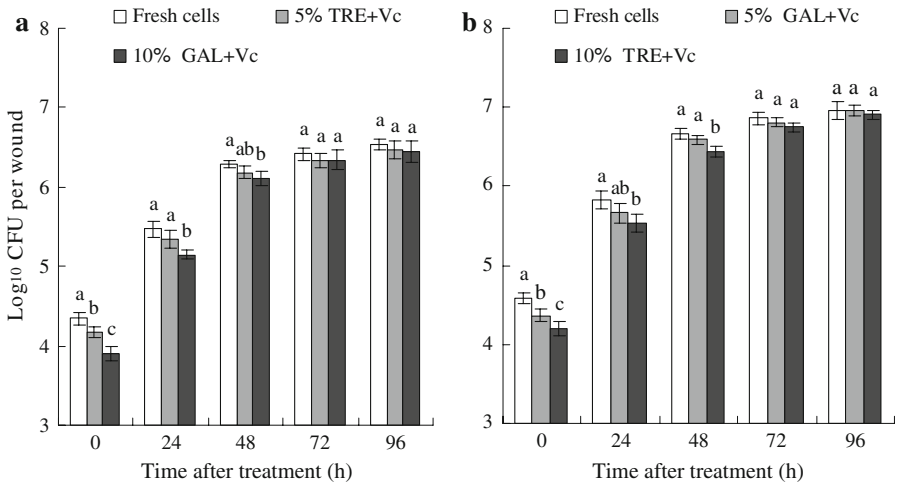

Fig. 2 Population dynamics of liquid formulations of *C. laurentii* (**a**) and *P. membranaefaciens* (**b**) after 90 days of storage at 4°C in wounds of peach fruits. Fruits were wounded and inoculated with 20 µl of formulated or fresh cells of *C. laurentii* or *P. membranaefaciens* at 1×10^7 CFU ml^{-1}. Samples for population measurement were assessed every 24 h for 96 h. Yeast colonies were counted after incubation at 26°C for three days and expressed as the Log_{10} CFU per wound. Error bars indicate standard deviations. Columns with different letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$). Abbreviations: TRE, trehalose; GAL, galactose

48 h after inoculation (Fig. 2). Such period is important for germination and infection of *P. expansum*. This may explain why *C. laurentii* and *P. membranaefaciens* in 5% TRE + Vc and 5% GAL+Vc liquid formulations with high cell viability provided good control of *P. expansum* on peach fruits, respectively (Fig. 1). The results demonstrated that biocontrol efficacy of the two yeasts was maintained after formulation and storage and depended on the number of viable inoculated cells. This was consistent with the results obtained in *P. anomala* by Melin et al. (2006).

Moreover, it was found that storage temperature had great influence on cell survivals of the two yeasts in liquid formulation. The number of viable counts was much higher at 4°C than at 25°C (Tables 1 and 2). In a previous study, it was also demonstrated that after freeze drying, the viability of *C. laurentii* was much higher when stored at 4°C than at 25°C (Li and Tian 2007). It is well known that low temperature can make the metabolic activity of microbial agents at a low level, which is beneficial to keep a high viability (Aguilera et al. 2007). However, it was noticed that 10% galactose and combining it with Vc maintained high viabilities >60% of *P. membranaefaciens* after 15 days at 25°C (Table 2), indicating that galactose-based liquid formulation may be promising for *P. membranaefaciens* under short-term storage at room temperature.

In conclusion, we first reported liquid formulations of *C. laurentii* and *P. membranaefaciens* and found that viability of the two yeasts in liquid formulation was improved by applying sugar protectant combined with Vc, and their biocontrol efficiency was maintained well after 90 days of storage at 4°C. The results of this study are promising to develop a formulation of the two antagonists suitable for commercial application.

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References

Abadias M, Benabarre A, Teixidó N, Usall J, Viñas I (2001) Effect of freeze drying and protectants on viability of the biocontrol yeast *Candida sake*. Int J Food Microbiol 65:173–182

Abadias M, Usall J, Teixidó N, Viñas I (2003) Liquid formulation of the postharvest biocontrol agent *Candida sake* CPA-1 in isotonic solutions. Phytopathology 93:436–442

Aguilera J, Randez-Gil F, Prieto JA (2007) Cold response in *Saccharomyces cerevisiae*: new functions for old mechanisms. FEMS Microbiol Rev 31:327–341

Ahmad S, Anzar A, Srivastava AK, Srivastava PK (2005) Effect of curing, antioxidant treatment, and smoking of

buffalo meat on pH, total plate count, sensory characteristics, and shelf life during refrigerated storage. Int J Food Prop 8:139–150

Crowe JH, Carpenter JF, Crowe LM (1998) The role of vitrification in anhydrobiosis. Annu Rev Physiol 60:73–103

Droby S, Cohen L, Daus A, Weiss B, Horev B, Chalutz E, Katz H, Keren-Tzur M, Shachnai A (1998) Commercial testing of Aspire: a yeast preparation for the biological control of postharvest decay of citrus. Biol Control 12:97–101

Eleutherio EC, Araujo PS, Panek AD (1993) Role of the trehalose carrier in dehydration resistance of *Saccharomyces cerevisiae*. Biochem Biophys Acta 1156:263–266

Fan Q, Tian SP (2001) Postharvest biological control of grey mold and blue mold on apple by *Cryptococcus albidus* (Saito) Skinner. Postharvest Biol Technol 21:341–350

Helbig J (2002) Ability of the antagonistic yeast *Cryptococcus albidus* to control *Botrytis cinerea* in strawberry. BioControl 47:85–99

Jakubowski W, Biliński T, Bartosz G (2000) Oxidative stress during aging of stationary cultures of the yeast *Saccharomyces cerevisiae*. Free Radic Biol Med 28:659–664

Janisiewicz WJ, Jeffers SN (1997) Efficacy of commercial formulation of two biofungicides for control of blue mould and grey mould of apples in cold storage. Crop Prot 16:629–633

Li BQ, Tian SP (2006) Effects of trehalose on stress tolerance and biocontrol efficacy of *Cryptococcus laurentii*. J Appl Microbiol 100:854–861

Li BQ, Tian SP (2007) Effect of intracellular trehalose in *Cryptococcus laurentii* and exogenous lyoprotectants on its viability and biocontrol efficacy on *Penicillium expansum* in apple fruit. Lett Appl Microbiol 44:437–442

Li BQ, Zhou ZW, Tian SP (2008) Combined effects of endo- and exogenous trehalose on stress tolerance and biocontrol efficacy of two antagonistic yeasts. Biol Control 46:187–193

Lima G, De Curtis F, Castoria R, De Cicco V (2003) Integrated control of apple postharvest pathogens and survival of biocontrol yeasts in semi-commercial conditions. Eur J Plant Pathol 109:341–349

Melin P, Håkansson S, Eberhard TH, Schnürer J (2006) Survival of the biocontrol yeast *Pichia anomala* after long-term storage in liquid formulations at different temperatures, assessed by flow cytometry. J Appl Microbiol 100:264–271

Melin P, Håkansson S, Schnürer J (2007) Optimisation and comparison of liquid and dry formulations of the biocontrol yeast *Pichia anomala* J121. Appl Microbiol Biotechnol 73:1008–1016

Nunes C, Usall J, Teixidó N, Viñas I (2001) Biological control of postharvest pears diseases using a bacterium, *Pantoea agglomerans* CPA-2. Int J Food Microbiol 70:53–61

Patiño-Vera M, Jiménez B, Balderas K, Ortiz M, Allende R, Carrillo A, Galindo E (2005) Pilot-scale production and liquid formulation of *Rhodotorula minuta*, a potential biocontrol agent of mango anthracnose. J Appl Microbiol 99:540–550

Piano S, Neyrotti V, Migheli Q, Gullino ML (1997) Biocontrol capability of *Metschnikowia pulcherrima* against *Botrytis* postharvest rot of apple. Postharvest Biol Technol 11: 131–140

Salek AT, Arnold WM (1995) Trehalose-stabilisation of osmophilicity and viability of baker's and distiller's yeast: applications to storage and drying. Chem Mikrobiol Technol Lebensm 17:14–24

Sampedro JG, Uribe S (2004) Trehalose-enzyme interactions result in structure stabilization and activity inhibition. The role of viscosity. Mol Cell Biochem 256:319–327

Singer MA, Lindquist S (1998) Thermotolerance in *Saccharomyces cerevisiae*: the Yin and Yang of trehalose. Trends Biotechnol 16:460–468

Spadaro D, Gullino ML (2004) State of the art and future prospects of the biological control of postharvest fruit diseases. Int J Food Microbiol 91:185–194

Teixidó N, Usall J, Palou L, Asensio A, Nunes C, Viñas I (2001) Improving control of green and blue molds of oranges by combining *Pantoea agglomerans* (CPA-2) and sodium bicarbonate. Eur J Plant Pathol 107:685–694

Torres R, Usall J, Teixidó N, Abadias M, Viñas I (2003) Liquid formulation of the biocontrol agent *Candida sake* by modifying water activity or adding protectants. J Appl Microbiol 94:330–339

Xu XB, Tian SP (2008) Reducing oxidative stress in sweet cherry fruit by *Pichia membranaefaciens*: a possible mode of action against *Penicillium expansum*. J Appl Microbiol 105:1170–1177

Xu XB, Qin GZ, Tian SP (2008) Effect of microbial biocontrol agents on alleviating oxidative damage of peach fruit subjected to fungal pathogen. Int J Food Microbiol 126: 153–158

Yao HJ, Tian SP, Wang YS (2004) Sodium bicarbonate enhances biocontrol efficacy of yeasts on fungal spoilage of pears. Int J Food Microbiol 93:297–304

Yu T, Wang LP, Yin Y, Feng FQ, Zheng XD (2008) Suppression of postharvest blue mould of apple fruit by *Cryptococcus laurentii* and N^6-benzyladenine. J Sci Food Agr 88:1266–1271

Zhang HY, Zheng XD, Xi YF (2005) Biological control of postharvest blue mold of oranges by *Cryptococcus laurentii* (Kufferath) Skinner. BioControl 50:331–342

Zhang HY, Wang L, Ma LC, Dong Y, Jiang S, Xu B, Zheng XD (2009) Biocontrol of major postharvest pathogens on apple using *Rhodotorula glutinis* and its effects on postharvest quality parameters. BioControl 48:79–83